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Analysis of urinary prostacyclin and thromboxane/prostacyclin ratio in patients with rheumatoid arthritis using gas chromatography/selected ion monitoring

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Summary We investigated production of prostacyclin and the urinary ratio of thromboxane and prostacyclin in patients with rheumatoid arthritis. The prostacyclin production level was assessed according to the level of urinary 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ measuring by gas chromatography/selected ion monitoring. In patients receiving medication, the prostacyclin level was lower and the thromboxane/prostacyclin ratio was greater compare with that of healthy volunteers. The prostacyclin level in patients without medication was approximately 4-fold higher than that of healthy volunteers and 8-fold higher than those of medicated groups. Although the ratio of the group without medication was similar to that of healthy volunteers, the urinary levels of each prostanoid were higher than those of other groups. Then, the ratios of groups receiving steroids were higher than that of other groups owing to high TX level. The present findings demonstrated that endogenous prostacyclin and thromboxane production increased in patients without medication, and prostacyclin production decreased with medication. © 2001 Harcourt Publishers Ltd

INTRODUCTION

Rheumatoid arthritis (RA) is a disease that causes inflammation at various joints in the whole body, and the joint is destroyed by abnormal multiplication of synoviocytes. In the inflammatory joint, cyclooxygenase (COX)-2 is induced in the synoviocyte, cartilage and inflammatory monocyte,[1,2] and various prostanoids such as prostaglandin (PG) E_2, PGF_2, prostacyclin (PGI_2) and thromboxane (TX) are released from the synoviocyte and other tissue.[3–5] PGE_2 is related to not only inflammation but also joint destruction by stimulating bone resorption.[6,7] The role and production levels of other prostanoids in RA patients remains unclear. In a previous study, we determined the urinary level of 11-dehydro-TXB_2, the major urinary metabolite of TXA_2, in RA patients and showed that the levels of TX were higher than that of healthy volunteers and altered by medication and surgery.[8]

Recently, it was reported that arachidonic acid is preferentially metabolized to PGI_2 and PGE_2 by COX-2 in rat peritoneal macrophages.[9] Furthermore, the action of PGI_2 during inflammation has been extensively investigated since a study demonstrated that mice lacking prostacyclin receptor (IP receptor) differed from normal mice in pain perception and inflammatory response.[10] These suggested that prostacyclin plays an important role in inflammatory diseases.

In the present study, we investigated the endogenous production level of PGI_2 in the same urine sample of RA patients used in our previous study of TX

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production levels.[8] The PGI_2 production was assessed by measuring urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ using gas chromatography/selected ion monitoring (GC/SIM). The 2,3-dinor-6-keto-$PGF_{1\alpha}$, the major urinary metabolite of PGI_2, has been suggested to indicate the physiological role of the parent compound and to be useful to assess PGI_2 production in whole body.

Furthermore, the stimulation of neulocyte migration and aggregation as part of the inflammatory reaction has often observed in clinical medicine. The synthetic balance of TX as platelet aggregated agent and PGI_2 as anti-aggregated agent is very important for diseases in blood vessels. Therefore, we investigated the TX/PGI_2 ratio in order to assess endogenous production of these prostanoids in inflammation and RA patients.

PATIENTS AND METHOD

Materials

Standard materials of 2,3-dinor-6-keto-$PGF_{1\alpha}$ and its deuterio derivative ([3,3,4,4-*d*]2,3-dinor-6-keto-$PGF_{1\alpha}$) were purchased from Cyman Chemicals Co. (Ann Arbor, MI, USA). The Sep-Pak C18 cartridge was purchased from Waters Associates (Milford, USA). Bond-Elut Si was purchased from Varian (Harbor City, USA). Dimethylisopropylsilyl (DMIPS)-imidazole was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide. Other solvents and reagents were the highest quality available.

Patients and healthy volunteer

Urine was obtained from 32 RA patients and nine healthy volunteers as controls. Before beginning this test, we obtained informed consent from all subjects. The diagnosis of RA was established according to the criteria of the American College of Rheumatology in 1987. The characteristics of the patients are shown in Table 1. All patients, except for five, had been treated with drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), steroids, disease-modifying antirheumatic drugs (DMARD), and other drugs.

Table 1 Patient characteristics

Number of patients	32 (Male: 6, Female: 26)
Age (years)	62 ± 12
Stage*	I: 4, II: 1, III: 3, IV: 19
Class*	I: 9, II: 10, III: 8, IV: 5
WBC ($\times 10^3$ cells/μl)	7.1 ± 2.6
PLT ($\times 10^5$ cells/μl)	3.0 ± 1.1
CRP (mg/dl)	3.8 ± 2.7
ESR (mm/h)	77.5 ± 37.0

*The stage and class were classified according to criteria of the American College of Rheumatology in 1987.

Healthy volunteers with a mean age of 29 years (range 23–35) had taken no medication for at least 2 weeks prior to urine collection.

Sample preparation

Human urine collected from healthy volunteers and RA patients was stored at −80°C until use.

The purification and derivatization of 2,3-dinor-6-keto-$PGF_{1\alpha}$ was according to the simplified method of Mizugaki et al.[11] Each urine sample (10 ml) was supplemented with 2,3-dinor-6-keto-$PGF_{1\alpha}$-d_4 (2.5 ng) as an internal standard (IS). The extraction of 2,3-dinor-6-keto-$PGF_{1\alpha}$ was derived to the spirolactone form with acidification to pH 3 with hydrochloride solution and used in the ODS column. The acidified urine was applied to a Sep-Pak C18 cartridge, which was then washed with water (10 ml) and *n*-hexane (10 ml). The prostanoid fraction was eluted with ethyl acetate (10 ml) and evaporated under reduced pressure. The residue was purified using silica gel column on ethyl acetate-*n*-hexane (1:5) (10 ml) and then the fraction of 2,3-dinor-6-keto-$PGF_{1\alpha}$ was evaporated under reduced pressure. The residue reacted with 1% methoxyamine hydrochloride in pyridine at 60°C for 1 h. After the pyridine was removed with an N_2 stream, the residue was treated with ethereal diazomethane. Finally, 2,3-dinor-6-keto-$PGF_{1\alpha}$ and IS as a sample for GC/SIM were derived to the methyl ester-methoxyamine-DMIPS ether derivatives by the treatment of DMIPS-imidazole and the excess reagent was removed by Bond Elut Si with ethyl acetate-*n*-hexane (2:98) (10 ml).

Measurement of urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$

Quantitation was performed using GC/SIM in the positive electron impact ionization mode. The GC/MS system was an HP5890 gas chromatography (Hewlett Packard, USA) and DX303 mass spectrometer (JEOL, Japan) equipped with an MP65HT (QADREX, USA) fused silica capillary column (25 m × 0.25 mm i.d.). The oven temperature was programmed from 200°C to 320°C (8°C/min), and the injector at 320°C. The carrier gas was helium. The ionization energy and accelerating voltage were 70 eV and 3 kV, respectively. The resolution of the mass spectrometer was at 1000. GC/SIM analysis was monitored using the characteristic ion of $[M-43]^+$ derived from elimination of the isopropyl radical. Thus, the derivatives of 2,3-dinor-6-keto-$PGF_{1\alpha}$ and IS were monitored at *m/z* 642.4 and *m/z* 646.4, respectively.

Creatinine contents

Creatinine in human urine was determined using a Creatinine test kit (Wako Pure Chemical Industries, Osaka, Japan). The results are shown as corrected values.

Statistical calculations

Statistical analysis was performed using StatView software (Abacus Concepts Inc., Berkeley, CA, USA). Student's unpaired *t*-test and Mann–Whitney's *U*-test were used to analyse the findings. Differences were considered significant at $P<0.05$.

RESULTS

Urinary level of 2,3-dinor-6-keto-$PGF_{1\alpha}$ in RA patients

We determined the urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ in human volunteers (age: 29 ± 5) and RA patients (age: 62 ± 12) (Table 1). The level in healthy volunteers was 46.0 ± 41.6 pg/mg creatinine and that of RA patients receiving medication was 22.8 ± 14.5 pg/mg creatinine ($P<0.05$ versus healthy volunteers as shown in Fig. 1). Five patients without medication, the untreated patients, showed very high levels of 2,3-dinor-6-keto-$PGF_{1\alpha}$ (160.3 ± 131.9 pg/mg creatinine) which was approximately 4-fold higher than that of healthy volunteers ($P<0.05$). One untreated patient who had an 2,3-dinor-6-keto-$PGF_{1\alpha}$ level of 52.1 pg/mg creatinine, showed normal levels for other laboratory data [erythrocyte sedimentation rate (ESR): 45 mm/h, C-reactive protein (CRP): 0.35 mg/dl). Pathologic diagnosis from photographs of knee synoviocytes indicated rheumatism (photograph not shown). Furthermore, the significant difference between that of RA patients receiving and not receiving medication was $P<0.001$.

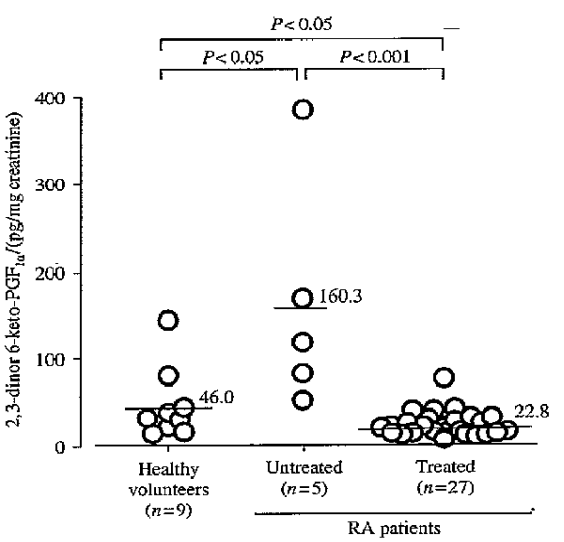

Fig. 1 Urinary levels of 2,3-dinor-6-keto-$PGF_{1\alpha}$ in RA patients and healthy volunteers.

Effects of drugs on urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ levels

We examined the effects of various drugs administered to patients on the 2,3-dinor-6-keto-$PGF_{1\alpha}$ level (Fig. 2). The NSAIDs groups without steroids (C, D in Fig.2) and healthy volunteers showed significant differences ($P<0.05$). However, there were significant differences between the untreated group and all treated groups ($P<0.05$–0.005).

Urinary TX/PGI_2 ratio in RA patients

In a previous study, we demonstrated that urinary 11-dehydro-TXB_2 levels in same urine samples of these RA patients were very high compared with that of healthy volunteers.[8] The production balance of TX as a platelet aggregating agent and PGI_2 as an anti-aggregating agent is very important for diseases in blood vessels. In the present study, we investigated the urinary TX/PGI_2 ratio in these patients.

The TX/PGI_2 ratio of 27 RA patients receiving medication was 43.4 ± 43.0 (mean$\pm$SD), and that of healthy volunteers was 15.6 ± 12.1 (Fig. 3). The TX/PGI_2 ratio of RA patients was significantly higher than that of healthy volunteers as controls ($P<0.05$). The TX/PGI_2 ratio of the untreated group was similar to that of healthy volunteers.

Effects of drugs on the TX/PGI_2 ratio

The patients were classified into seven groups according to the drugs administered (Fig. 4). The TX/PGI_2 ratio of the untreated group was similar to that of healthy volunteers. As shown in Figure 5, each prostanoid level in the untreated group entirely differed from that of

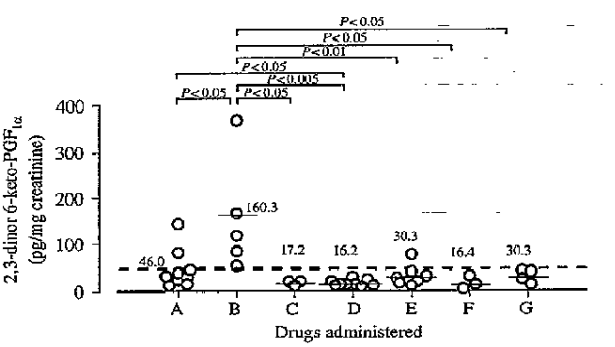

Fig. 2 Relationship of urinary levels of 2,3-dinor-6-keto-$PGF_{1\alpha}$ and drugs in RA patients; A: Healthy volunteers, B: Untreated, C: NSAIDs, D: NSAIDs+Others*, E: NSAIDs+Steroids, F: NSAIDs+Others+Steroids, G: Steroids.
*Others are DMARDs and other drugs for RA therapy. The broken line is the mean of the healthy volunteers.

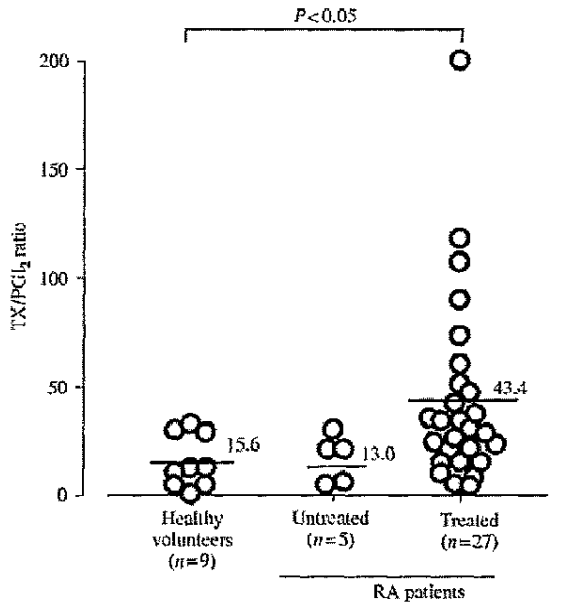

Fig. 3 Urinary TX/PGI_2 ratio in RA patients and healthy volunteers.

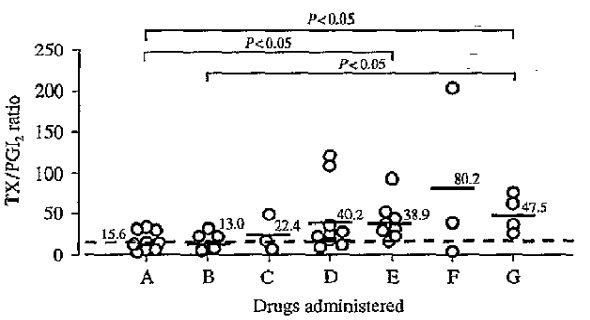

Fig. 4 Relationship of the TX/PGI_2 ratio and drugs in RA patients; A: Healthy volunteers, B: Untreated, C: NSAIDs, D: NSAIDs+Others*, E: NSAIDs+Steroids, F: NSAIDs+Others+Steroids, G: Steroids. *Others are DMARDs and other drugs for RA therapy. The broken line is the mean level of the healthy volunteers.

healthy volunteers. This finding demonstrated that these prostanoids in the untreated group were increased in production. Then, the TX/PGI_2 ratio of the groups receiving steroids were larger than those of healthy volunteers and the untreated group. Since the level of 2,3-dinor-6-keto-$PGF_{1\alpha}$ in the groups receiving steroids were slightly lower than that of healthy volunteers, it was suggested that these ratios were larger depending on the TX level. In addition, the TX/PGI_2 ratio of the groups receiving NSAIDs showed slightly larger ratios compared with that of healthy volunteers by inhibition of production of both prostanoids.

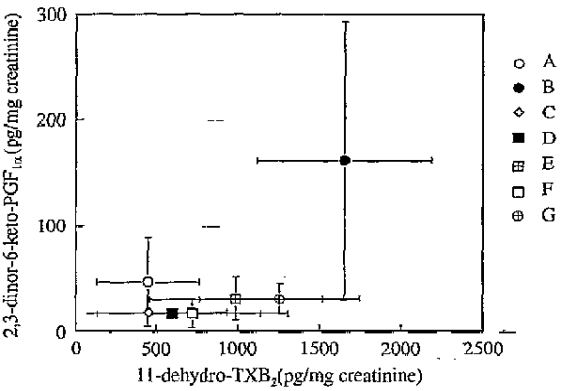

Fig. 5 Effects of drug on the TXB_2 and PGI_2 contents in RA patients; A: Healthy volunteers, B: Untreated, C: NSAIDs, D: NSAIDs+Others*, E: NSAIDs+Steroids, F: NSAIDs+Others+Steroids, G: Steroids. *Others are DMARDs and other drugs for RA therapy.

Relationship between urinary prostanoid levels and laboratory data

We investigated the relationships between urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ and the TX/PGI_2 ratio, and the laboratory data using RA diagnosis as an indicator. As shown in Table 2, there were no correlations between the level of 2,3-dinor-6-keto-$PGF_{1\alpha}$ and the white blood content (WBC), platelet content (PLT), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), respectively, in all patients. However, the correlation coefficients of this prostanoid and the laboratory data, except for CRP, in the untreated group tended to be higher than that of patients receiving medication. There was no relationship between the TX/PGI_2 ratio and the laboratory data in any patient.

DISCUSSION

In inflammatory joints, various prostanoids are released from synoviocytes, and other inflammatory cells. It was reported that synovial cells, chondrocytes and synovium microvessel endothelial cells released PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ by stimulating with IL-1 in vitro.[5,12] Furthermore, the joint fluid collected from RA patients contained not only those prostanoids but also TXB_2.[3] In a previous study, the level of PGE_2 in the joint fluid collected from RA patients was higher than that of osteoarthritis patients as controls.[13] In addition, the urinary level of 11-dehydro-TXB_2 in RA patients was higher than that of healthy volunteers.[8] In those studies, it was shown that the production of each prostanoid was enhanced.

In the present study, we measured urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ levels instead of prostacyclin in RA patients using gas chromatography/selected monitoring and it

Table 2 Correlation coefficients (r) of prostanoids and laboratory data in RA patients

	TX			PGI_2			TX/PGI_2		
	All patients (n=32)	Untreated (n=5)	Treated (n=27)	All patients (n=32)	Untreated (n=5)	Treated (n=27)	All patients (n=32)	Untreated (n=5)	Treated (n=27)
WBC	0.086*	0.281	0.129	0.181	0.487	0.062	0.515	0.158	0.541
PLT	0.047	0.452	0.116	0.281	0.625	0.010	0.375	0.283	0.357
CRP	0.224	0.827	0.035	0.331	0.099	0.105	0.126	0.028	0.013
ESR	0.039	0.508	0.033	0.076	0.508	0.060	0.257	0.204	0.231

*Coefficient index.

appeared that the level in untreated patients was approximately 4-fold higher than that of healthy volunteers and that of groups receiving medication. Moreover, the TX/PGI_2 ratio of RA patients was higher than healthy volunteers even though the RA patients were receiving medication.

The high levels of urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ in the untreated group demonstrated that production of PGI_2 had increased in those patients. As many studies have suggested, PGI_2 is released from synoviocytes and other inflammatory cells, and is contained in joint fluid collected from RA patients as described above.[3,5,12] Furthermore, the transient increase in PGI_2 was observed in acute inflammatory model rats with adjuvant arthritis[14] and with carrageenin-induced pleurisy.[15] These findings suggested that PGI_2 played an important role in inflammation. It is interesting that endogenous production of PGI_2 increased not only these acute inflammatory models[14,15] but also in RA as a chronic inflammatory disease.

The medication appeared to influence the urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ level (Fig. 1). The level in patients receiving medication was significantly lower than that of healthy volunteers ($P<0.05$) and that of patients without medication ($P<0.001$). When RA patients were classified into six groups based on drugs administrated, the level of all groups receiving medication such as NSAIDs and steroids for RA therapy were lower than healthy volunteers (Fig. 2). There were significantly differences between the levels of the medication groups and the untreated group ($P<0.05$–0.005). These findings indicated that production of PGI_2 decreased by medication. NSAIDs directly inhibit the production of PGH_2 in COX. Steroids not only inhibit phospholipase A_2, but also down-regulate the expression of COX-2 mRNA by decreasing the expression of IL-1 in synovium microvessel endothelial cells obtained from RA patients.[12,16] Although the biological mechanism of steroids in inflammation is versatile and unclear, these findings in vitro agree with the present observation in vivo. The determination of urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ levels appears to be useful to evaluate the effect of medication.

In previous studies, both of the level of PGE_2 in joint fluid and that of urinary 11-dehydro-TXB_2 in RA patients receiving steroids were higher than those of the group receiving NSAIDs.[8,13] It is interesting that 2,3-dinor-6-keto-$PGF_{1\alpha}$ levels are different from those of other prostanoids during medication. In addition, the urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ levels in diabetes and retinal occlusion tended to be similar or slightly lower compared with those of healthy volunteers.[17,18] The present findings in RA patients also differed from the findings in these other diseases.

Furthermore, we examined the urinary TX/PGI_2 ratio of PGI_2 and TX[8] in the same urine samples of these patients (Fig. 3). The TX/PGI_2 ratio of RA patients receiving medication was significantly higher than healthy volunteers ($P<0.05$). However, the ratio of the untreated group was similar to that of healthy volunteers. The urinary levels of each prostanoid in this group was higher than that of other groups (Fig. 5). In this group, it was demonstrated that each prostanoid production was increased. When RA patients were classified into six groups according to medication, the ratio of many patients receiving NSAIDs was similar to that of healthy volunteers (Fig. 4). As shown in Figure 5, both the levels of TX and PGI_2 in these groups were similar to those of healthy volunteers, which indicated that the production of these prostanoids was inhibited by NSAIDs. However, the ratios in the groups receiving steroids were larger than that of healthy volunteers (Fig. 4). In these groups, it appeared that the PGI_2 production was depressed by steroids as described above.[12,16] However, the TX levels in these groups were similar to that of the untreated group and higher than that of the healthy volunteers. Although it is unclear what cell type primarily produces these prostanoids, one previous study reported findings supported by the present observations. Seppala et al. reported that NSAIDs diminished the production of PGE_2, $PGF_{2\alpha}$, PGI_2 and TXB_2 in an investigation using rheumatic synovial cell primary culture in vitro. The hydrocortisone diminished the production of PGI_2 by about 80% of NSAIDs as the controls, however, the TXB_2 production was almost unaltered.[19]

In addition, the correlation coefficients of the PGI_2 level and laboratory data in the untreated group tended to be large compared with that of patients with medication as shown in Table 2. However, the relationship of the PGI_2

level and CRP were different from the relationship of TX and the laboratory data. This indicates that changes in the urinary PGI_2 level may reflect the condition of this disease although the mechanism is unclear. The low correlation coefficients of the TX/PGI_2 ratio and the laboratory data was caused by high levels of each prostanoid in this group.

In the present study, it appeared that the urinary PGI_2 level in RA patients without medication was significantly higher than that of healthy volunteers, and the PGI_2 levels in RA patients receiving medication were lower than that of healthy volunteers. The TX/PGI_2 ratio in RA patients was significantly higher than that of healthy volunteers. In previous study, we suggested the possibility that the urinary 11-dehydro-TXB_2 level was a useful marker of diagnosis and therapy in RA. The urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ level may also be a useful marker of diagnosis and medication in RA. The use of urine is useful not only to assess PGI_2 production in the whole body but also avoids suffering of patients.

Recently, one study reported that mice lacking prostacyclin receptor and normal rats showed differences in pain perception and inflammatory response,[10] and the action of PGI_2 during inflammation subsequently received more attention. The action and behavior of PGI_2 in RA is still unclear. The present findings may be helpful to clarify a role of PGI_2 in RA.

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REFERENCES

1. Sano H., Hla T., Maier J. A. M., et al. In vivo cyclooxygenase expression in synovial tissues of patients with rheumatoid arthritis and rats with adjuvant and streptococcal cell wall arthritis. *J Clin Invest* 1992; **89**: 97–108.
2. Crofford L. J., Wilder R. D., Ristimaki A. P., et al. Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. *J Clin Invest* 1994; **93**: 1095–1101.
3. Trang L., Granstrom E., Lovgren O. Levels of prostaglandins $F_{2\alpha}$ and E_2 and thromboxane B_2 in joint fluid in rheumatoid arthritis. *Scand J Rheumatology* 1977; **6**: 151–154.
4. Higgs G. A., Moncada S., Salmon J. A., Seager K. The source of thromboxane and prostaglandins in experimental inflammation. *Br J Pharmacol* 1983; **79**(4): 863–868.
5. Knott I., Dieu M., Burton M., Lecomte V., Remacle J., Reas M. Differential effects of interleukin 1α and β on the arachidonic acid cascade in human synovial cells and chondrocytes in culture. *Agents and Actions* 1993, **39**: 126–131.
6. Robinson D. R., Tashjian H., Levine L. Prostaglandin-stimulated bone resorption by rheumatoid synovia. *J Clin Invest* 1975; **56**: 1181–1188.
7. Bergmsn P., Schoutens A. Prostaglandins and bone. *Bone* 1995; **16**(4): 485–488.
8. Nakamura H., Hishinuma T., Sawai T., et al. Analysis of the urinary 11-dehydro thromboxane in rheumatoid arthritis patients using gas chromatography/selected ion monitoring. In submission.
9. Brock T. G., McNish R. W., Peters G. M. Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E_2. *J Biol Chem* 1999; **274**(17): 11660–11666.
10. Murata T., Ushikubo F., Matsuoka T., et al. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* 1997; **388**(6643): 678–682.
11. Mizugaki M., Hishinuma T., Yu Grace S.P., et al. Microanalysis of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ in human urine using gas chromatography/high resolution-selected ion monitoring. *J Chromatogr* 1994; **658**(1): 11–19.
12. Szcepanski A., Moatter T., Carley W. W., Gerritsen M. E. Induction of cyclooxygenase II in human synovial microvessel endothelial cells by interleukin-1: inhibition by glucocorticoids. *Arthritis Rheum* 1994; **37**(4): 495–503.
13. Hishinuma T., Nakamura H., Sawai T., Uzuki M., Itabashi Y., Mizugaki M. Microdetermination of prostaglandin E_2 in joint fluid in rheumatoid arthritis patients using gas chromatography/selected ion monitoring. *Prostaglandin and other Lipid Mediators* 1999; **58**: 179–186.
14. Stichenoth D. O., Selve N., Tsikas D., Gutzki F.-M., Frolich J. C. Increased total body synthesis of prostacyclin in rats with adjuvant arthritis. *Prostaglandins* 1995; **50**: 331–340.
15. Harada Y., Tanaka K., Uchida Y., et al. Changes in the levels of prostaglandins and thromboxane and their roles in the accumulation of exudate in rat carrageenin-induced pleurisy – a profile analysis using gas chromatography–mass spectrometry. *Prostaglandins* 1982; **23**(6): 881–895.
16. Masferrer J. L., Seibert K. Regulation of prostaglandin synthesis by glucocorticoids. *Receptor* 1994; **4**(1): 25–30.
17. Hishinuma T., Koseki Y., Murai Y., Yamazaki T., Suzuki K., Mizugaki M. Urinary thromboxane A_2/prostacyclin balance reflects the pathological state of a diabetic. *Prostaglandins & Other Lipid Mediators* 1999; **58**: 263–271.
18. Yamanobe S., Nakagawa Y., Hishinuma T., Mizugaki M., Tamai M. Analysis of urinary 11-dehydro thromboxane B_2 in patients with occluded retinal vein using GC/SIM. *Prostaglandins Leukotrienes and Essential Fatty Acids* 1998; **58**: 65–68.
19. Seppala E. Production of prostanoids by rheumatic synovial cells in vitro: effects of anti-inflammatory drugs on arachidonic acid metabolism. *Clin Rheumatol* 1987; **6**(2): 170–176.